Distribution and Variation of a Collagen-Degrading Enzyme in the Uterus of Pregnant Rats

In the involutionary uterus of humans[1], as well as of rats[2,3], a collagen-degrading factor was found. In the non-pregnant rats, its activity is low[4,5]. It increases mainly during involution of the uterus after parturition. The cause of this mechanism, for degrading the collagen of the uterus which had increased during gravidity, is unknown.

Rats, and other small rodents, develop on the outside of the uterus at the site of each placenta, during the second half of the pregnancy, a so-called 'glandula myometralis'. The function of these organs is unknown. BLOCH suggested, on the basis of histological studies, that they perforate the uterus musculature towards the end of pregnancy and liberate catabolic enzymes[6].

We compared the collagen-degrading activity of extracts of the glandulae myometrales with that of extracts from the uterus wall during pregnancy and post partum. In this way, the role of the glands may be analysed.

Experimental. We used 20 Wistar rats from the 11th day of pregnancy (earliest time at which the glandulae can be prepared) until 150 h post partum. The animals are killed by a blow and the uterus dissected. The glandulae and the remaining uterus are separately weighed and immediately frozen.

At 0–4°C, homogenates are then prepared by a Waring blendor in a solution containing 0.05% acetic acid, 0.5% NaCl, 0.1% Triton-X-100 and some drops of toluene and octanol. On aliquots the N content is measured by the Kjeldahl procedure. The homogenates are centrifuged at 3000 *g* for 30 min and the residues discarded.

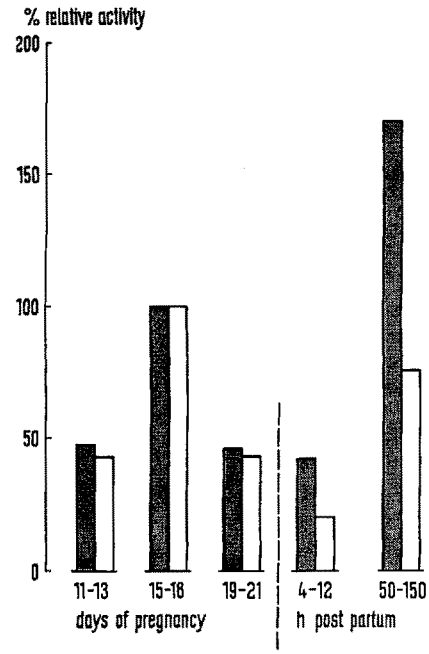
The enzyme-containing supernatants (in a final concentration of 0.5–1.0%) are incubated with collagen at 38°C and pH 3.3. After 15–40 min the enzymic reaction is stopped by adding ice-cooled sulphosalicylic acid (final concentration 5%). The samples are left overnight in the cold and then centrifuged. After acid hydrolysis (15 h at 110°C in 4 *N* HCl) of the supernatant hydroxyproline, the characteristic amino acid of collagen, is measured colorimetrically[7].

As substrate acid-soluble collagen from tail tendons of 5-month-old rats in Michaelis buffer[8] (pH 3.3, ionic strength 0.15) is used. Blanks are incubated with buffer alone. The specific activity of the enzyme extracts is expressed in μg hydroxyproline liberated per min and per mg N of the original homogenate.

To compare the kinetic properties of the collagen-degrading enzyme from the uterus wall with that from the glandula, we determined its affinity to the substrate by calculating the Michaelis constants (Km) plotting the results after the equation of HANES[9].

Results. The total N content of 1% homogenates remains fairly constant during the second half of pregnancy with 0.220 mg N/ml ($\sigma \pm 0.025$) for the glandula and with 0.252 mg N/ml ($\sigma \pm 0.024$) for the uterus. The Figure shows the time-course of the activities in arbitrary units taking the highest values of both tissues during pregnancy as 100%. During involution the uterus activity rises to 168%, while that of the glandula falls to 76%. The specific activities of the uterus wall for the 5 periods given in the Figure are 2.55, 5.43, 2.71, 2.23, and 9.07, respectively. Those of the glandula are 4–8 times higher. The ratios of the activities of the glandula to those of the uterus are 7.1, 8.9, 7.1, 4.0, and 3.6, respectively.

The collagen-degrading activities of homogenates of the same concentration from the uterus wall and from the glandula were separately determined. Then the homogenates were also mixed in order to examine whether the difference of their activities is caused by specific effectors. The Table shows that such effectors are hardly involved. The activities, however, in the mixture of both homogenates are always smaller than expected (mean −6.2%). Therefore the presence of an inhibitor in the uterus wall during pregnancy cannot be excluded.



Collagen – degrading activity of uterus wall extract (black) and glandula myometralis extract (white).

Influence of tissue effectors on the collagen degradation *in vitro*

Final concentration of enzyme extracts	Activity during pregnancy: ΔE_{562}/10 min/sample					
	1	2	3	4	5	6
U/2	0.088	0.158	0.238	0.281	0.140	0.228
G/2	0.126	0.240	0.419	0.496	0.512	0.466
(U + G)/2 Expected	0.214	0.398	0.657	0.777	0.652	0.694
Found	0.186	0.393	0.565	0.758	0.651	0.652
Difference in %	—13.1	—1.3	—14.0	—2.4	—0.2	—6.1

U = uterus extract, G = glandula myometralis extract.

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The kinetic properties of the two enzyme extracts are significantly different. The smaller Michaelis constant of the uterus wall (Km = 0.0627%, $\sigma \pm 0.0072$) signifies that the enzymic composition of the uterus wall has a greater affinity to collagen than that of the glandula (Km = 0.212%, $\sigma \pm 0.013$).

In the involutionary period, an extract from the glandula is still 4 times more active than an uterus extract. But the weight of the uterus is 6–10 times higher than that of the glandulae. Thus the total activity of the uterus surpasses that of the glandulae. Moreover, the affinity of the uterus enzyme to collagen is 3 times greater.

These results confirm the high enzymic activity of the glandula myometralis during pregnancy and show an accumulation of the collagen-degrading enzyme in the uterus wall during involution. It cannot yet be decided whether the collagen-degrading enzyme in the involutionary uterus is secreted by the glandulae and activated in the uterus, or whether it originates from the stroma of the uterus wall itself[10].

Zusammenfassung. Extrakte aus Uteruswand und Glandula myometralis bauen bei saurem pH Kollagen ab. Die Drüsen haben eine grössere spez. Aktivität. Doch zeigt das Uterusenzym eine grössere Affinität zum Substrat, und seine Gesamtaktivität überwiegt post partum.

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Bestimmung des zeitlichen Verlaufes der Erschlaffung der kontraktilen Elemente mit Hilfe des Spannungs-Frequenz-Diagrammes

Für die Bestimmung des aktiven Zustandes eines Muskels sind eine Reihe von Methoden entwickelt worden, die meist einen relativ grossen Aufwand erfordern. Von HILL[1–3] ist die Methode der schnellen Dehnung (quick stretch) des Muskels angegeben worden, die über die Änderung des Dehnungswiderstandes des aktivierten Muskels den zeitlichen Verlauf und das Ausmass der Kontraktion der kontraktilen Elemente festlegen lässt.

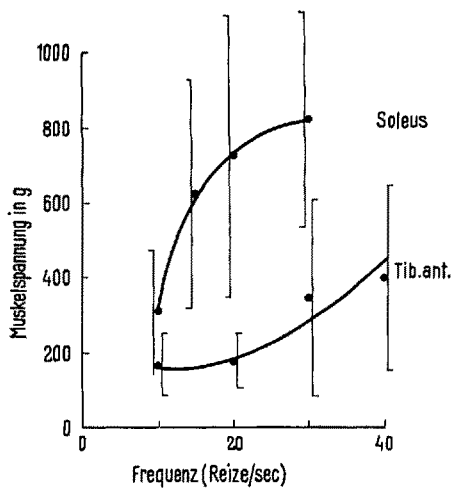

Fig. 1. Spannungs-Frequenzkurven des Soleus und Tibialis anterior. Ordinate: Spannungsentwicklung in g. Abszisse: Reizfrequenz in Impulsen/sec. Die Punkte sind Mittelwerte von durchschnittlich 70 Bestimmungen. Die Streuung ist als Standardabweichung vom Mittelwert angegeben. Sie ist deshalb sehr gross, weil das Gewicht der Muskeln sehr stark variierte (das Gewicht der Katzen lag zwischen 1,5 und 4,0 kg).

Die Plateaudauer des aktiven Zustandes kann mit Hilfe tetanischer Reizung erfasst werden, entweder durch Bestimmung der Verschmelzungsfrequenz[4], oder durch Messung der Zeit zwischen dem letzten Reiz eines Tetanus und dem Beginn des Spannungsabfalles im Mechanomyogramm[5]; oder aber durch Superposition der Spannungskurven nach einem Einzel- und einem Doppelreiz und Messung der Zeit zwischen Reiz und Trennung der Spannungskurven[6]. Eigene vergleichende Untersuchungen[7] ergaben, dass der Methode der Superposition der Spannungskurven der Vorzug zu geben ist.

Die abfallende Phase des aktiven Zustandes schliesslich, die der Erschlaffung der kontraktilen Elemente entspricht, lässt sich mit der Methode der schnellen Entlastung (quick release[8–10]) ermitteln.

Geht man von der Überlegung aus, dass Muskelzuckungen sich nur dann summieren, wenn das Reizintervall kürzer als die Gesamtdauer des aktiven Zustandes ist[11], und dass die maximale tetanische Spannungsentwicklung der maximalen Kontraktion der kontraktilen Elemente gleichgesetzt werden kann[1], dann müsste sich der Verlauf der abfallenden Phase des aktiven Zustandes durch die Kurve der jeweils grössten Spannungssummation bei verschiedenen Reizfrequenzen ebensogut erfassen lassen wie mit der Methode der schnellen Entlastung.

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